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# Manganese Superoxide Dismutase (MnSOD) and Autoantibodies Against MnSOD in Acute Viral Infections

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Sera of 146 patients with acute EBV, HAV, HBV, CMV, HSV, and rubella virus infections, and sera from 35 healthy controls were tested for the antioxidant enzyme manganese superoxide dismutase (MnSOD). An enzyme immunoassay that detects all isomeres of the enzyme was developed. The mean MnSOD value of healthy controls was 107 ng/ml. In HAV, HBV and EBV infections characterized by viral replication in internal organs, there was an average 5-fold rise of serum MnSOD, whereas in viral infections with low direct cytopathogenity, such as rubella, CMV and HSV, the MnSOD levels showed only minor rises. These sera were also tested for autoantibodies against MnSOD using a novel sensitive indirect enzyme immunoassay. The average IgM anti-MnSOD concentration in sera of healthy controls was 112 GU. In sera of patients with acute HBV, CMV, HSV or rubella virus infections IgM anti-MnSOD values were only slightly raised above the cut-off level. In contrast, in some patients with acute EBV infections anti-MnSOD concentrations rose up to 20-fold of normal values. In HAV infections the same phenomenon was observed in patients who had reactivated EBV infections. These findings indicate that EBV may facilitate the B-cell response to MnSOD. These autoantibodies may inhibit the protective function of MnSOD and prolong the disease by oxygen injury. Our concept on the pathogenic effect of the autoantibodies against MnSOD emphasizes the importance of the antioxidant enzyme in viral infections. ***J. Med. Virol. 55:161–167, 1998.*** 

**KEY WORDS:** MnSOD; autoantibodies; EBV; pathogenesis; viral infections

## INTRODUCTION

Since the first description [McCord et al., 1969] increasing interest has been focused on superoxide dismutases, the scavengers of superoxide that are produced in biological systems during enzymatic and non enzymatic oxidations. In mammalian cells there are two superoxide dismutases, the copper-zinc type (Cu/ZnSOD) found mainly in the cytosol, and the manganese type (MnSOD) in the mitochondria. In extracellular fluids the constitutive Cu/ZnSOD seems to play a minor role with a half time of only a few minutes [Wong et al, 1989; Shingu et al, 1994], while MnSOD expression is inducable with a longer half-time of 6 hrs [Wong et al, 1989; Gorecki et al., 1991]. The concentration of MnSOD is high in organs with an elevated rate of respiration, such as liver, lung, and myocardium [Marklund, 1984]. Providing defense mechanisms against lipid peroxidation MnSOD is not only active in the cell, but it is also secreted into its surroundings in order to protect the outer cell membrane [Yoshida et al., 1994]. In local or systemic inflammation both the increased oxidative metabolism and cytokines induce MnSOD expression [Sato et al., 1995]. Thus, the host cell protects itself against the cytokine-induced oxidative burst that opposes the invading organisms [Roos, 1991].

Increased amounts of superoxide radicals are produced in diseases accompanied by massive cell destruction and a high rate of purine metabolism. Patients with acute or chronic leukaemia, polycythaemia vera, and pernicous anaemia have increased serum MnSOD levels [Nishiura et al., 1992; Taniguchi et al., 1992]. Many viral infections also induce cell and tissue destruction. In murine models influenza virus or cytomegalovirus induced pneumonitis lead to remarkable increases of intracellular and extracellular oxygen radicals [Akaike et al., 1990, Ikeda, et al., 1992] and cell damage due to oxidative stress is only prevented as long as sufficient amount of MnSOD is produced.

In humans the influence of acute viral infections on

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MnSOD concentrations in sera and the effect of the enzyme activity on the course of the disease are largely unknown. Recently, however, autoantibodies against MnSOD were detected in patients with infectious mononucleosis [Ritter et al., 1994] and therefore the significance of MnSOD in viral infections including acute EBV infections can be elucidated.

## METHODS AND MATERIAL

### Sera

Between 1992 and 1994 6,000 sera deriving from patients with viral diseases and healthy controls were examined for MnSOD and autoantibodies against MnSOD. From these MnSOD values and IgM anti-MnSOD values of 25 patients with infectious mononucleosis (mean age 34 yrs), 30 patients with CMV infections (mean age 38), 10 patients with acute HSV infections (mean age 22), 36 patients with rubella (mean age 24), 20 patients with acute HAV infections (mean age 41), and 28 patients with acute HBV infections (mean age 41) have been selected for this study; 35 healthy blood donors (mean age 35) served as controls.

### Isolation of MnSOD

MnSOD was isolated from 90 g human liver obtained at a post mortem from a patient, with no neoplastic or hepatic disease [Matsuda et al., 1991]. The protein concentration was determined routinely and the enzyme activity was measured in an indirect assay [McCord et al., 1969].

### Preparation of Specific Antibodies Against Anti-MnSOD

Hyperimmune serum against purified human MnSOD with a titre of 1:250,000 was made in a rabbit; IgG anti-MnSOD was separated by a CL-4B-anion exchange column (Pharmacia, Schweden).

### Enzyme-Linked Immunoassay for the Detection of MnSOD

Wells of the microtitre plates were coated with 0.8 $\mu$g of the purified rabbit IgG anti-MnSOD in 100 $\mu$l 50 mM acetate/citrate buffer, pH 5.5, incubating at 4°C for 16 hrs. 100 $\mu$l serum samples were added in dilutions of 1/20, 1/40, 1/80 in potassium phosphate buffer with 1% FKS and 0.1% Tween 20 (vol/vol/vol), pH 6.5. Horseradish peroxidase-conjugated rabbit IgG anti-MnSOD in the dilution 1:500 in 1% FKS/PBS (vol/vol) (PBS: 2 mM $KH_2PO_4$, 180 mM NaCl, 9 mM $Na_2HPO_4$, pH 7.2) was used for detection. The samples were incubated for 2 h at 37°C. Three washing steps were carried out with 0.05% Tween 20/PBS (vol/vol).

### Enzyme-Linked Immunoassay for the Detection of Antibodies to MnSOD

Wells of the microtitre plates were coated with 1.4 $\mu$g MnSOD in 100 $\mu$l 50 mM acetate/citrate buffer, pH 5.5, incubating at 4°C for 16 hrs. Postcoating was performed with 100 $\mu$l 20% FKS/PBS (vol/vol) per well. 100 $\mu$l serum was applied in the dilution 1/20, 1/40, 1/80 in potassium phosphate puffer containing 1% FKS and 0.1 % Tween 20 (vol/vol/vol) pH 6.5. Horseradish peroxidase conjugated anti-human-Ig/H/$\mu$ was used as the second antibody. Washing steps and incubation were carried out as described for the MnSOD detection assay. The serum of a patient with an anti-MnSOD-antibody titer of 1,000 GU (= Göttingen units) was used as a control.

### Serological Diagnosis of Different Viral Infections

Acute and reactivated EBV infections were detected as described elsewhere (Ritter et al., 1993). Other viral infections were diagnosed by commercially available tests as decribed previously [Ritter et al., 1996].

## RESULTS

### Concentration of MnSOD in Sera

The serum MnSOD values were measured in 35 healthy blood donors using an ELISA that detects all MnSOD isomeres (Fig. 1, Column VII). The average MnSOD level of this control group was 107 ng/ml, with a cut-off level at 130 ng/ml. Columns I to VI show MnSOD values of patients with acute viral infections. The average MnSOD concentration in 25 patients with infectious mononucleosis, the clinical presentation of acute EBV infection, was 376 ng/ml, representing a >3-fold rise compared to controls. Three of these patients suffered from protracted disease complicated by rupture of the spleen, haemolytic anaemia and severe hepatitis. In these patients MnSOD concentration rose to particulary high values (between 700 and 1300 ng/ml). The highest MnSOD values were measured in patients with acute hepatitis A (mean 683 ng/ml) and B (mean 615 ng/ml) virus infections (Fig. 1, Columns II, III).

MnSOD levels of patients with CMV and HSV infections, in contrast, were markedly lower with a mean value of 182 ng/ml, and 229 ng/ml, respectively (Fig. 1, Column IV, V). Patients with rubella had MnSOD concentrations that did not differ significantly from the controls (mean value 115 ng/ml in Fig. 1, Column VI).

### Concentration of IgM Anti-MnSOD in Sera

IgM anti-MnSOD concentrations were measured by an indirect ELISA that is more sensitive than the immunoblot technic used before. Serum IgM anti-MnSOD concentrations in healthy controls and from patients with acute viral infections are shown in Fig. 1 (Column I to VI). The average anti-MnSOD antibody level in sera of the 35 healthy blood donors was 112 GU (Fig. 2, Column VII). The cut-off level of IgM anti-MnSOD was fixed at 336 GU. Anti-MnSOD values in patients with acute EBV infections greatly vary; the average anti-MnSOD titre in acute EBV infections was 1369 GU, but in some individuals this value was almost 6-fold higher. These were all patients with complicated clinical courses. In patients with acute HAV infections high IgM anti-MnSOD concentrations and great variations were also seen. In contrast, only slightly elevated anti-

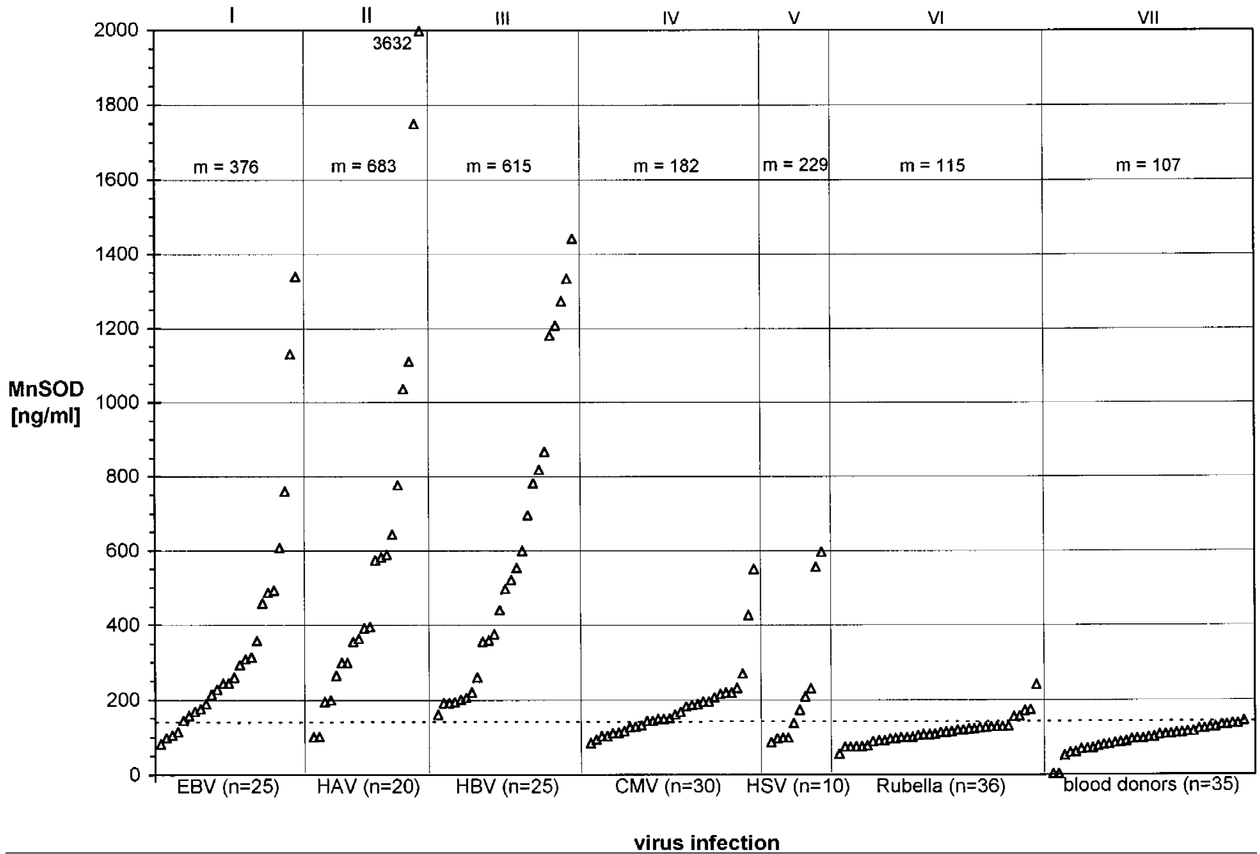


Fig. 1. Concentration of MnSOD in different viral infections.

MnSOD antibody values were found in HBV, CMV, HSV, and rubella virus infections.

### Comparing MnSOD and IgM Anti-MnSOD Concentrations in Different Viral Infections

Only patients with acute EBV or HAV infections had high autoantibody values to MnSOD (IgM anti-MnSOD <2000 GU in Fig. 2. Columns I, II), namely 4 out of 25 patients with acute EBV infection and 7 out of 20 patients with acute HAV infection. Serological investigations of the patients with HAV infection revealed that 9 patients had reactivated EBV infection. All of the 7 patients presenting with a marked rise of IgM anti-MnSOD belonged to this group.In the one patient who had HSV infection with raised MnSOD, and IgM anti-MnSOD values, reactivated EBV infection was also diagnosed. Nevertheless, in the twelve patients with acute HBV infections and high serum MnSOD values anti-MnSOD was not found in high serum concentrations. In these individuals EBV reactivation was not detected. Thus, marked elevations of anti-MnSOD were only observed in patients with acute or reactivated EBV infections.

## DISCUSSION

The pathogenesis of viral disease is caused by both the infectious agent and the immune reaction of the host. Recently, a new aspect of pathogenesis, the effect of superoxide, was suggested to play a role in viral infections, as it does in many other diseases such as reperfusion damage or autoimmune disease (McCord, 1983, Leff, 1994). In all these conditions superoxide levels are raised as a result of tissue destruction via a raised purine metabolism. Superoxide, in turn, causes cell injury that may lead to further destruction unless antioxidant enzymes are available in sufficient amounts for protecting the host. The failure to induce enzyme expression according to its demand has been reported to result in degeneration and cell death [Furuta et al., 1995; Thompson, 1995].

The study revealed that serum MnSOD can be raised in all viral infections, but in both HAV and HBV infections or in infectious mononucleosis MnSOD concentrations can be much higher when compared to non cytopathogenic viral infections like rubella. Infection with HSV and CMV show intermediate values associated with only minor cell destruction. In HSV infections the virus first replicates in the epithelium, where there is only local necrosis, and organ involvement occurs only later. In CMV infections, again, there may not be a marked cell destruction. Thus, in these patients MnSOD measured during follow-up investigations may be used as a marker for the progression of the disease. The validity of this concept has been shown previously in

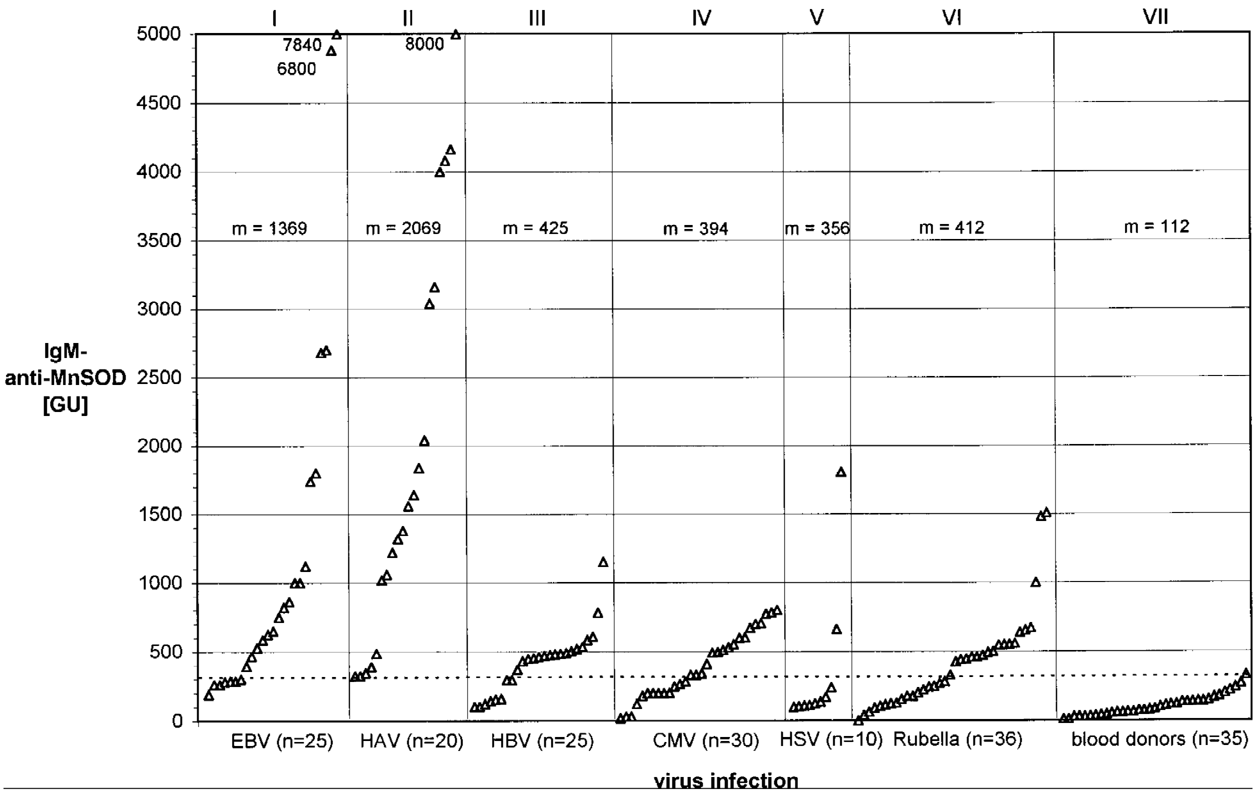


Fig. 2. Concentration of IgM anti-MnSOD in different viral infections.

bacterial disease, during adult respiratory distress syndrome due to sepsis where MnSOD was demonstrated to serve as a predictor of a high risk [Leff et al., 1993].

It is relevant to consider the different potential sources of increased serum MnSOD levels. These may rise as a result of tissue necrosis, in particular in viral infections involving organs with high concentrations of MnSOD, such as the liver. In addition to the release of MnSOD as a result of viral replication and direct cell lysis, increased serum MnSOD can also be due to cytokine actions generated during the immune responses to the virus (Fig. 3). Considering patients with acute EBV-infections marked cell destruction is indicated by increased levels of uric acid. Such an increased purine metabolism coincides with raised activity of the xanthine-oxidase which is known to lead to the increase of superoxide radicals. This process, that upregulates MnSOD expression and secretion of MnSOD into the periplasmatic space and to the circulation, is facilitated by cytokines (Sato et al., 1995). As the half-time of MnSOD is 6 hrs, an effective protection of the organism by antioxidant enzymes is warranted. Cu/ZnSOD, in contrast, is less effective as its half-time is shorter, and its activity is inhibited by hydrogen peroxide [Gandy et al., 1982].

As long as the concentration of superoxide radicals is regulated by the mechanisms indicated in Fig. 3, surrounding cells are protected against oxygen injury. In murine model experiments it has been documented that during viral infections of the myocardium and the lung, i.e. in the two organs with a high rate of respiration and high MnSOD concentrations, the MnSOD shows a protective role against superoxide injury [Suzuki et al., 1993; Akaide et al., 1990]. In murine pneumonitis induced by influenza virus and cytomegalovirus a correlation was found between an increased activity of the xanthine oxidase and the degree of pathological lesions in the lung [Akaide et al., 1990; Ikeda et al., 1992].

The down-regulation of MnSOD, with the consequent superoxide rise, may occur as a result of two different mechanisms. First, in the virus infected cells MnSOD may be down regulated along with the disruption of the host cell metabolism. Flores and coworkers observed a repression of MnSOD expression by the Tat protein in HIV infected cells [Flores et al., 1993] and the accumulation of superoxide radicals is assumed to be one of the initial steps in the pathogenesis of AIDS [Edeas et al., 1995].

A second mechanism that has been described by us is the inhibition of MnSOD by autoantibodies (see lower part of Fig. 3). In the original paper a relatively insensitive immunoblot technique was used and IgM anti-MnSOD was only detected in patients with acute EBV infections [Ritter et al., 1994]. When a more sensitive enzyme-linked immunoassay was developed in this work low levels of IgM anti-MnSOD were found in the sera of healthy controls as well. Thus, we presume that the autoantibodies present in low concentrations are natural antibodies which may regulate enzyme activity, as it has already been suggested in regard to other

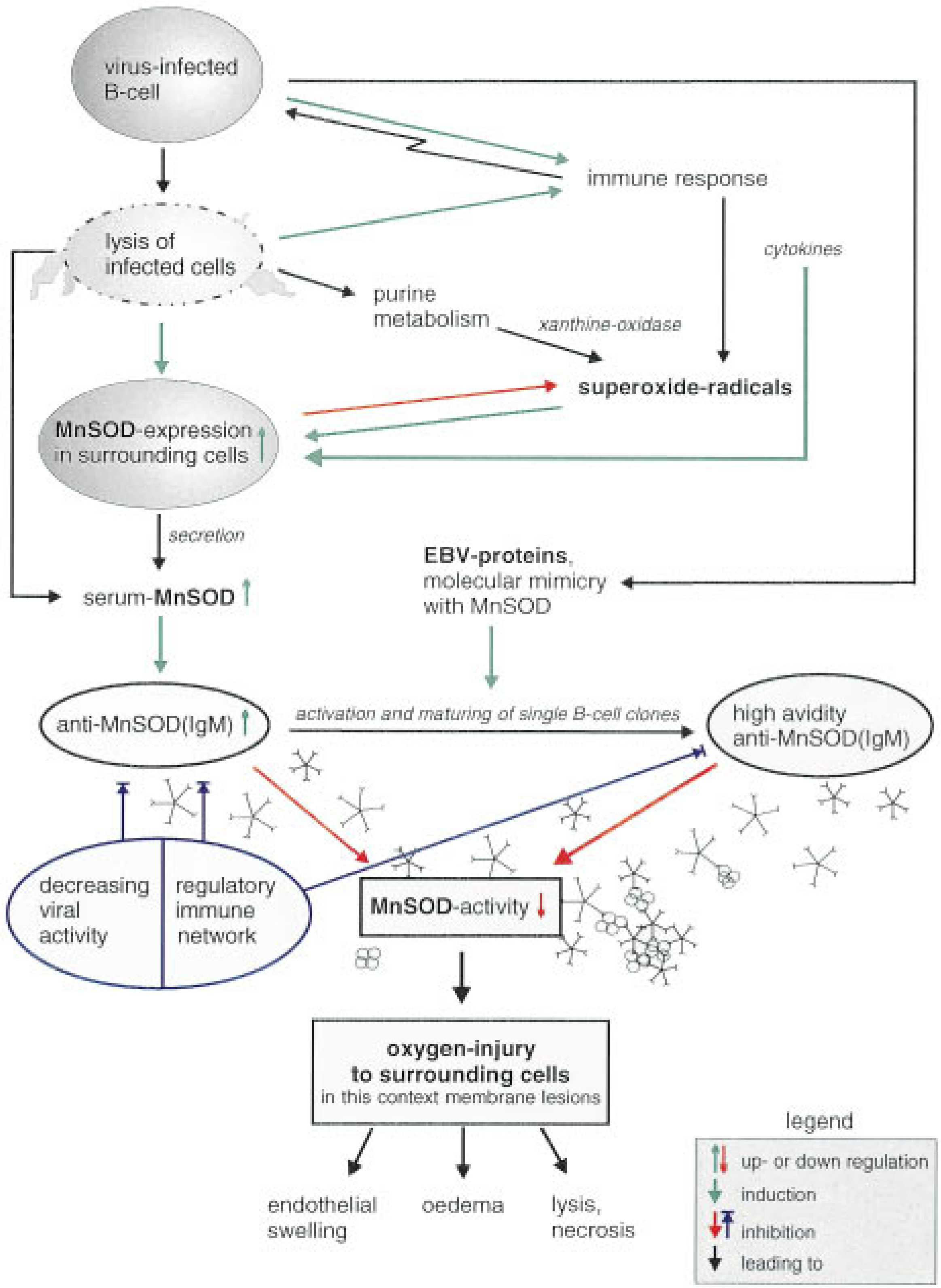


Fig. 3. Induction, release, and effect of MnSOD in acute viral infections and the pathogenic influence of autoantibodies against MnSOD.

enzymes [Avrameas et al., 1983]. Normally, the production of these naturally occuring autoantibodies is controlled by an idiotypic network [Varela et al., 1991].

This regulation may also be effective in the majority of acute viral infections where the observed elevated serum levels of MnSOD are accompanied by only minor rises of IgM anti-MnSOD. Thus, in patients with acute HBV infections even the dramatic rises of MnSOD are

associated with a meagre increase of IgM anti-MnSOD values to only twice the normal levels. A closer analysis of the results shown in this paper above reveals that the only exception to this rule of efficient autoantibody control is seen in patients infected with EBV and HAV. Very high titres of anti-MnSOD were observed in all patients with acute EBV infections who had high serum MnSOD concentrations. Interestingly, in patients with acute HAV infections, where this phenomenon was observed, reactivated EBV infection was found. This finding is often seen in hepatitis A infections [Ritter et al., 1996]. At the same time none of the patients with acute HBV infections had a reactivation of EBV. These findings taken together, indicate that EBV infection itself can over-rule the idiotypic controls of an antibody production against MnSOD. Indeed there is a molecular mimicry between EBNA1 and MnSOD and also between the BRLF1 protein of EBV and this enzyme [Dalpke et al., 1996]. We assume that the molecular mimicry does not only cause a general rise of the anti-MnSOD antibody titre, but the contact with EBNA1 and the BRLF1 protein may induce an increase of the affinity and avidity of the autoantibodies. A rise of the affinity of IgM autoantibodies via somatic hypermutation due to contact with an exogenous antigen has already been described for the the rheumatoid factor (Mantovani et al., 1993). Antibodies with a higher affinity and avidity are particulary effective in inhibiting the enzyme activity of MnSOD [Ritter et al., 1994]. Such a loss of enzyme activity leads to accumulation of superoxide radicals that induce cell injury (Fig. 3). The obvious consequences of these pathological events are the damaged endothelial function which leads to oedema and swelling of organs, to propagated injury of liver cells during hepatitis, and to mild haemolysis due to erythrocyte damage. In acute HAV and EBV infections these complications are well known, some time they present in serious, even life threatening forms [Semrau et al., 1996]. We suggest that these complications are caused by an autoimmune inhibition of MnSOD due to a disturbed regulary network. Patients can recover when viral replication decreases and the regulary network is restored.

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